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Detection of malaria in goats and sheep

Deteksi malaria pada kambing dan domba

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Plasmodium species, as the causative agent of malaria, are a significant concern in livestock production. This study aims to investigate the presence of malaria parasites in small ruminants comprised of goats and sheep. *Plasmodium* infections in ungulates do not cause malaria in humans. However, they might have anopheline mosquitoes as the same vector of this parasite. This study uses molecular techniques, nested PCR, to detect *Plasmodium* infections in small ruminants, as traditional microscopic examination may lead to misdiagnosis. The results show that Plasmodium infections were found in goat samples from Sumba Barat Daya, Nusa Tenggara Timur, with a prevalence of 7.41% based on nested PCR assay. Notably, *Plasmodium* was not detected through microscopic examination, underscoring the sensitivity of molecular techniques. It should be noted that none of the goats tested positive for malaria based on microscopic examination, suggesting extremely low parasitemia levels. This study emphasizes the importance of understanding *Plasmodium* infections in small ruminants, shedding light on their impact on animal health and their potential role in local transmission patterns. The sample size in this study is limited. Further research with larger samples and expanded geographical scope is recommended to comprehensively understand *Plasmodium* prevalence in small ruminants.

Kata kunci:
Kambing
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A B S T R A K

Plasmodium merupakan parasit darah penyebab malaria dan menjadi perhatian penting dalam produksi ternak. Penelitian ini bertujuan untuk menyelidiki keberadaan parasit malaria pada ternak ruminansia kecil yaitu kambing dan domba. Infeksi Plasmodium pada kambing dan domba tidak menyebabkan malaria pada manusia. Namun, mereka memiliki vektor yang sama yaitu nyamu Anopheles. Penelitian ini menggunakan metode molekuler yaitu nested PCR, untuk mendeteksi infeksi Plasmodium pada ternak ruminansia kecil. Hal ini dilakukan karena pemeriksaan mikroskopis secara konvensional dapat menyebabkan salah diagnosa. Hasil penelitian menunjukkan bahwa infeksi Plasmodium ditemukan pada sampel kambing dari Sumba Barat Daya, Nusa Tenggara Timur, dengan prevalensi 7,41% berdasarkan uji nested PCR. Pada penelitian ini, Plasmodium tidak terdeteksi melalui pemeriksaan mikroskopis, yang menguatkan bahwa metode molekukler lebih sensitive. Kambing yang dinyatakan positif terkena malaria berdasarkan pemeriksaan molekuler tidak teramati adanya gejala klinis, menunjukkan tingkat parasitemia yang sangat rendah. Penelitian ini menekankan pentingnya memahami infeksi Plasmodium pada ternak ruminansia kecil, yang memberikan wawasan tentang dampaknya pada

kesehatan hewan dan peran potensi dalam persebaran parasit ini. Jumlah sampel dalam penelitian ini terbatas, sehingga penelitian lebih lanjut dengan sampel yang lebih besar dan cakupan geografis yang lebih luas direkomendasikan untuk memahami secara komprehensif prevalensi Plasmodium pada ternak ruminansia kecil.

INTRODUCTION

Blood parasites are considered one of the primary causes of disruptions in livestock production worldwide (Armour et al., 1996). These parasites pose a serious economic threat and remain a major concern for animals in tropical and subtropical regions (Soulsby, 1986) (Soulsby, 1986). Diseases caused by protozoa in the blood also result in significant losses for wild and domestic animals worldwide (Ozubek et al., 2020). *Plasmodium* is one such blood parasite that affects domestic animals, and it is also found in humans, causing a disease known as malaria.

Plasmodium species affecting animals, particularly ungulates, consist of various species. *Plasmodium bubalis* was first identified in water buffaloes in India (Sheather, 1919) and later confirmed in Thailand (Nugraheni et al., 2022). *Plasmodium traguli* has been found in mouse deer in Malaysia (Garnham & Edeson, 1962). *Plasmodium limnotragi* in Marshbuck (Tragelaphus spekii) in Africa (Van Den Berghe, 1937), *Plasmodium odocoilei* in White-tailed Deer (*Odocoileus virginianus*) in North America (Guggisberg et al., 2018; Martinsen et al., 2016), *Plasmodium brucei* and *Plasmodium cephalophi* in Duiker Antelope in Africa (Boundenga et al., 2016) and *Plasmodium caprae* in domestic goats (*C. aegagrus hircus*) in Africa (Mello & Paes, 1923). Further molecular assay has been conducted to identify these parasites (Martinsen et al., 2016; Templeton et al., 2016) and they have been found in several countries, including Zambia, Kenya, Sudan, Iran, Myanmar, and Thailand (Kaewthamasorn et al., 2018).

The economic impact *of P. caprae* as a cause of malaria in goats is generally unknown in goat farming. Research on *P. caprae* is limited, and malaria in goats typically does not exhibit clinical symptoms, with most cases presenting as low parasitemia (Kaewthamasorn et al., 2018). Recent studies have reported that goats infected with malaria may exhibit symptoms such as fever, tachycardia, tachypnea, and mucosal jaundice. Still, these observations have yet to be confirmed due to the presence of other concurrent infections (Al-Badrani & Alabadi, 2021).

The prevalence of malaria in goats is reported to be relatively low. Molecular detection of malaria in goats in Thailand showed positive results only in Phetchaburi province and not in Chonburi, Rayong, or Nan provinces. The gradual examination of *P. caprae* positivity in samples in Phetchaburi was reported as five positives out of 126 samples (4%), 0 positives out of 88 samples (0%), and 5 positives out of 100 samples (5%). The prevalence of *P. caprae* in goats in Thailand is sporadic, ranging from 0% to 5%, and is much lower than *P. bubalis* in water buffaloes, which ranges from 16% to 45% (Asada et al., 2018; Templeton et al., 2016).

Specific research on malaria in goats in Indonesia has not been conducted to date. Parasites resembling human *Plasmodium* in goats in malaria-endemic areas for humans have been reported in West Sumba, Fakfak (Munirah et al., 2020) and Kaligesing (Sumanto et al., 2021). These reports do not specify the possible blood parasite species found based on an approach to *P. caprae* since they focus on malaria affecting humans.

It is important to conduct investigations into how malaria parasites affect the transmission dynamics in ungulates, including their impact on the health of both animals and humans. By studying ecosystems, we can develop strategies to control and prevent malaria. Hence, this study aims to contribute to understanding *Plasmodium* infections in small ruminants. In this study, malaria parasites were found and screened in goats and sheep using nested PCR, which targets the *cytochrome b oxidase* (cytb) gene. The results of this investigation will improve our knowledge of Plasmodium infections in ruminants. Provide valuable insights into the broader epidemiological and evolutionary context of the malaria parasites in small ruminants. The findings from this study could have implications for controlling and preventing malaria among small ruminants.

MATERIALS AND METHODS

Ethical statements

The protocol for this study was reviewed and approved by the Universitas Gadjah Mada, Faculty of Veterinary Medicine's Institutional Animal Care and Use Committee (IACUC), with the reference number 006/EC-FKH/Int./2023. This study obtained official veterinary government consent to collect blood samples from goats at defined places.

Sample collection and sampling site selection

A cross-sectional study was conducted to detect the Plasmodium parasite in goats from Nusa Tenggara Timur and Jember. The sampling location was chosen because this area has been documented to have probable human-endemic malaria. Each goat's blood was collected in three milliliters and immediately transferred to an ethylenediaminetetraacetic acid (EDTA) tube for preservation. Following sample collection, the samples were transferred to the Faculty of Veterinary Medicine laboratory at Universitas Gadjah Mada, utilizing an ice box to keep the temperature stable. Morphological and molecular approaches were used to investigate all samples.

Blood smear examination

Blood smears were taken for morphological analysis. Each blood smear was fixed in methanol for 10 minutes before being stained for 45 minutes with 10% Giemsa. The blood smears were then cleaned with clean water and allowed to dry. A drop of immersion oil was applied to each blood smear before being examined under an Olympus DP12 microscope at 1000x magnification. If at least one parasite was found in the blood smear, the sample was termed positive. Following World Health Organization criteria, at least 2,000 red blood cells (RBCs) were counted (WHO, 2015).

DNA extraction and nested PCR assay

Each whole blood sample (200 µL) in EDTA was extracted using the GeneJET Genomic DNA Purification Kit (Thermo Scientific™, USA) according to the manufacturer's protocol. Genomic DNA samples were then subjected to nested PCR for malaria screening using published primers DW2 (TAATGCCTAGACGTATTCCTGATTATCCAG) and DW4 (TGTTTGCTTGGGAGCTGTAAT-CATAATGTG) and nested primers NCYBINF (TAAGAGAATTATGGAGTGGATGGTG) and NCYBINR (CTTGTGGTAATTGACATCCAATCC), targeting 822 bp of the partial *cytochrome b oxidase* (*cytb*) gene, as previously conducted (Perkins & Schall, 2002; Templeton et al., 2016).

A total amount of 12.5 µL was used for the PCR experiment. A master mix containing 0.25 µL of KOD FX Neo Polymerase (Toyobo, Japan), 6.25 µL of 2X PCR Buffer, 2.5 µL of 2 mM dNTPs, 0.3 µM of each primer, and 1.75 µL of sterile distilled water (SDW) were included in each reaction. Each reaction also contained 1 µL of genomic DNA as a template. The initial PCR result was diluted ten times with SDW before being subjected as a DNA template in nested PCR (1 µL).

The PCR method required 40 cycles of denaturation at 98 °C for 10 s and annealing and extension at 62 °C for 3 min, with the first step being a 2-minute denaturation step at 94 °C. A single cycle at 68 °C for 5 minutes was then used for the final extension phase. Nested PCR was done under the same conditions. A mouse culture of *Plasmodium berghei* was used in this investigation as a positive control, while sterile distilled water (SDW) was used as a negative control. A total of 5 µL of PCR products were electrophoresed for 45 minutes on a 1.5% gel of agarose using 1x TAE at 120 volts and 400 mA.

Data analysis

The binomial Clopper-Pearson confidence interval was used to determine the percentage of samples with positive results from the nested PCR assay. An online statistical analysis tool is available at https://epitools.ausvet.com.au was used to conduct statistical analysis.

RESULTS AND DISCUSSION

In this study, *Plasmodium* sp. stadium was not observed based on microscopic examination. However, natural infection of *Plasmodium* sp. was detected in 4 samples from Sumba Barat Daya, Nusa Tenggara Timur based on PCR assay targeting mitochondrial DNA (Kb_Sb2, Kb_Sb4, Kb_Sb32, Kb_Sb38). The statistical analysis results indicate that the proportion of samples infected by *Plasmodium* sp. in Sumba Barat Daya was calculated as 0.0741 (95% CI: 0.0206-0.1789), whereas Jember (95% CI: 0.0000-0.0860 as presented in Table 1.

Plasmodium infection was found in goats in Sumba Barat Daya, Nusa Tenggara Timur,

Table 1. The estimated proportion of Plasmodium infection based on PCR assay was calculated by the binomial Clopper-Pearson confidence interval method

Location	Host	Number of tested	Number positive	Proportion (%)	95 % CL
SBD	Goat	54	4	0.0741	0.0206-0.1789
Jember	Sheep	41	0	0.0000	0.0000-0.0860
Total		95	4	0.0421	0.0116-0.1043

CL: confidence limits, SBD: Sumba Barat Daya

with 7.41% of examined samples giving positive results. In concordance with the study by Opara and Nwokedi (2011), the finding of this study implies that *Plasmodium* parasites are likely present in small ruminants in this area. However, no *Plasmodium* sp. was detected through microscopic examination, highlighting the superior sensitivity of molecular techniques like PCR in finding these parasites even when they are not readily visible using blood smear examination methods. It is also crucial to note that none of the goats tested positive for malaria, showing that the parasitemia level in these goats is exceedingly low. This finding is consistent with (Nguyen et al., 2023) recent study.

Plasmodium discovery in goats and sheep is crucial not only for understanding its impact on animal health but also for potential human health consequences. While *Plasmodium* species that infect ungulates do not directly cause malaria in humans, they do contribute to the general epidemiology of these parasites by acting as potential reservoirs and offering insight into local transmission patterns. As reported previously by (Opara & Nwokedi, 2011), *Plasmodium* sp. infections have been documented in sheep and goats, with reported prevalence rates reaching as high as 56.3%. Remarkably, these infections have been associated with a decline in packed cell volume (PCV), indicative of anemia, as noted previously by Aseme et al. (2020). The study also noted that prevalence rates of 12.0% and 36.0% for *Plasmodium* sp. infections have been detected in goats at the Trans Amadi and Rumuokoro abattoirs, respectively. Additionally, none of the sheep were detected for malaria infection. This result in contrast to the previous finding by Al-Badrani and Alabadi (2021) which noted that they found malaria parasite in sheep. The prevalence of *Plasmodium* in goats in areas with a history of human-endemic malaria necessitates additional research into potential zoonotic transmission routes and the involvement of these animals in the malaria ecosystem.

This study has taken a step for malaria detection in small ruminants by PCR assay in domestic goats and sheep, similar to the study by Opara and Nwokedi (2011). Nevertheless, one of the limitations of this study is the small sample size for malaria detection in small ruminants. Therefore, increasing the sample size and expanding the geographical scope might provide a more comprehensive knowledge of *Plasmodium* prevalence in small ruminants in Indonesia. Advanced research is necessary to identify the *Plasmodium* species found in goats and to investigate the genetic diversity of these parasites. Such further advanced research would shed light on evolutionary links and the mechanics of potential cross-species transmission.

CONCLUSIONS

Overall, the findings of this study reveal that natural malaria infections were found in Sumba Barat Daya area based on nested PCR assay. The study further revealed that malaria parasite infection in goats was asymptomatic. This suggests that natural malaria infection in small ruminants presenting with no clinical signs may require a sensitive tool for malaria diagnosis. PCR technology could increase the sensitivity of malaria detection in small ruminants since all the animals in this study were asymptomatic, and parasite load could be extremely low parasitemia level for microscopy. Additionally, since human malaria was reported to have a high prevalence in this area, malaria infection in domestic goats may associated with the presence of anopheline mosquitoes as the vector as well as breeding sites in the sample collection area. Therefore, advanced research on malaria in small ruminants is needed in order to develop malaria prevention and control strategies in animals. Further research on malaria

in small ruminants can provide insights into potential cross-species transmission dynamics as well as potential implications for human health.

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REFERENCES

Al-Badrani, B. A., & Alabadi, B. H. (2021). A preliminary Study of Malaria infection (Plasmodium spp.) in Iraqi Livestock. *Egyptian Journal of Veterinary Sciences*, *52*(1), 97-111.

Armour, J., Duncan, J. L., Dunn, A. M., Jennings, F. W., & Urquhart, G. M. (1996). *Veterinary Parasitology*. B-Blackwell Science, Wiley. https://books.google.co.id/books/about/Veterinary_Parasitology.html?id=sEl5QgAACAAJ&redir_esc=y

Asada, M., Takeda, M., Tomas, W. M., Pellegrin, A., de Oliveira, C. H. S., Barbosa, J. D., da Silveira, J. A. G., Braga, E. M., & Kaneko, O. (2018). Close relationship of *Plasmodium* sequences detected from South American pampas deer (*Ozotoceros bezoarticus*) to *Plasmodium* spp. in North American white-tailed deer. *Int J Parasitol Parasites Wildl*, *7*(1), 44-47. https://doi.org/10.1016/j.ijppaw.2018.01.001

Aseme, T., Robert, B., Amuzie, C., & Akani, G. (2020). Haematological Parameters and Haemoparasites of West African Dwarf Goats Sold at Trans-Amadi and Rumuokoro Abattoirs, Port Harcourt, Nigeria [Research article]. *Current Trends in Veterinary and Dairy Research*, *1*(1), 14-20.

Boundenga, L., Makanga, B., Ollomo, B., Gilabert, A., Rougeron, V., Mve-Ondo, B., Arnathau, C., Durand, P., Moukodoum, N. D., Okouga, A.-P., Delicat-Loembet, L., Yacka-Mouele, L., Rahola, N., Leroy, E., Ba, C. T., Renaud, F., Prugnolle, F., & Paupy, C. (2016). Haemosporidian Parasites of Antelopes and Other Vertebrates from Gabon, Central Africa. *PLoS One*, *11*(2), e0148958. https://doi.org/10.1371/journal.pone.0148958

Garnham, P., & Edeson, J. (1962). Two new malaria parasites of the Malayan mousedeer. *Rivista di malariologia*, *41*(1/3), 1-8.

Guggisberg, A. M., Sayler, K. A., Wisely, S. M., & Odom John, A. R. (2018). Natural History of Plasmodium odocoilei Malaria Infection in Farmed White-Tailed Deer. *Msphere*, *3*(2). https://doi.org/10.1128/mSphere.00067-18

Kaewthamasorn, M., Takeda, M., Saiwichai, T., Gitaka, J. N., Tiawsirisup, S., Imasato, Y., Mossaad, E., Sarani, A., Kaewlamun, W., Channumsin, M., Chaiworakul, S., Katepongpun, W., Teeveerapunya, S., Panthong, J., Mureithi, D. K., Bawm, S., Htun, L. L., Win, M. M., Ismail, A. A., Kaneko, O. (2018). Genetic homogeneity of goat malaria parasites in Asia and Africa suggests their expansion with domestic goat host. *Scientific Reports*, *8*(1), 5827. https://doi.org/10.1038/s41598-018-24048-0

Martinsen, E. S., McInerney, N., Brightman, H., Ferebee, K., Walsh, T., McShea, W. J., Forrester, T. D., Ware, L., Joyner, P. H., Perkins, S. L., Latch, E. K., Yabsley, M. J., Schall, J. J., & Fleischer, R. C. (2016). Hidden in plain sight: Cryptic and endemic malaria parasites in North American white-tailed deer (*Odocoileus virginianus*). *Science Advances*, *2*(2), e1501486. https://doi.org/10.1126/sciadv.1501486

Mello, F. d., & Paes, S. (1923). Sur une plasmodiae du sang des chèvres. Cr. *séanc. Soc. Biol.*, *88*, 829-830.

Munirah, M., Wahyuni, S., Wahid, I., & Hamid, F. (2020). Human Plasmodium in Domestic Animals in West Sumba and Fakfak, Indonesia.

Nguyen, A. H. L., Pattaradilokrat, S., Kaewlamun, W., Kaneko, O., Asada, M., & Kaewthamasorn, M. (2023). Myzomyia and Pyretophorus series of Anopheles mosquitoes acting as probable vectors of the goat malaria parasite Plasmodium caprae in Thailand. *Scientific Reports*, *13*(1), 145.

Nugraheni, Y. R., Arnuphapprasert, A., Nguyen, T. T., Narapakdeesakul, D., Nguyen, H. L. A., Poofery, J., Kaneko, O., Asada, M., & Kaewthamasorn, M. (2022). Myzorhynchus series of Anopheles mosquitoes as potential vectors of Plasmodium bubalis in Thailand. *Scientific Reports*, *12*(1), 5747.

Opara, M., & Nwokedi, C. (2011). Occurrence of haemoparasites among small ruminants reared under traditional husbandry system in Owerri, Southeast Nigeria. *Animal Health and Production*, *59*, 393-398.

Ozubek, S., Bastos, R. G., Alzan, H. F., Inci, A., Aktas, M., & Suarez, C. E. (2020). Bovine Babesiosis in Turkey: Impact, Current Gaps, and Opportunities for Intervention. *Pathogens*, *9*(12), 1041. https://doi.org/doi: 10.3390/pathogens9121041

Perkins, S. L., & Schall, J. J. (2002). A molecular phylogeny of malarial parasites recovered from cytochrome b gene sequences. *J Parasitol*, *88*(5), 972-978. https://doi.org/10.1645/0022-3395(2002)088[0972:Ampomp]2.0.Co;2

Sheather, A. (1919). A malaria parasite in the blood of a buffalo. *Journal of Comparative Pathology and Therapeutics*, *32*(223), 80026-80027.

Soulsby, E. J. L. (1986). *Helminths, Arthropods & Protozoa of Domesticated Animals*. Lea & Febiger. https://books.google.co.id/books/about/Helminths_arthropods_and_protozoa_of_dom.html?id=dN01XwAACAAJ&redir_esc=y

Sumanto, D., Hadisaputro, S., Sakundarno, A. M., Susanti, S., & Sayono. (2021). Human-Plasmodium like in domestic-goat blood in malaria endemic areas in Purworejo Indonesia. *Journal of Communicable Diseases (E-ISSN: 2581-351X & P-ISSN: 0019-5138)*, *53*(4), 148-152.

Templeton, T. J., Asada, M., Jiratanh, M., Ishikawa, S. A., Tiawsirisup, S., Sivakumar, T., Namangala, B., Takeda, M., Mohkaew, K., Ngamjituea, S., Inoue, N., Sugimoto, C., Inagaki, Y., Suzuki, Y., Yokoyama, N., Kaewthamasorn, M., & Kaneko, O. (2016). Ungulate malaria parasites. *Scientific Reports*, *6*, 23230. https://doi.org/10.1038/srep23230

Van Den Berghe, L. (1937). Plasmodium limnotragi n. sp. d'un antelope Limnotragus spekei. *Bull. Soc. Path. Exot*, *30*, 272-274.